

CLAIMS

We Claim:

1. An isolated alphavirus which infects human dendritic cells, with the proviso that said alphavirus is not ATCC # VR-2526.
2. An isolated alphavirus which infects non-human dendritic cells, with the proviso that said alphavirus is not a Venezuelan equine encephalitis virus or ATCC # VR-2526.
3. The isolated alphavirus according to claims 1 or 2 wherein said alphavirus is a Sindbis virus.
4. The isolated alphavirus according to claim 3 wherein said alphavirus has an amino acid substitution at E2 residue 160, as compared to wild-type Sindbis virus.
5. The isolated alphavirus according to claims 1 or 2 wherein said alphavirus is a Semliki Forest virus.
6. The isolated alphavirus according to claims 1 or 2 wherein said alphavirus is ATCC No. VR-2643.
7. An isolated nucleic acid molecule, comprising a nucleic acid molecule which encodes an alphavirus according to claims 1 or 2.
8. The nucleic acid molecule according to claim 7 wherein said alphavirus is a Sindbis virus.

9. The nucleic acid molecule according to claim 8 wherein said nucleic acid molecule encoding an alphavirus is shown in Figure 2B.

10. An isolated nucleic acid molecule, comprising a nucleic acid molecule which encodes an alphavirus as shown in Figure 2C

11. An alphavirus structural protein expression cassette, comprising a promoter operably linked to a nucleic acid sequence encoding alphavirus structural proteins from an alphavirus according to any one of claims 1 to 6.

12. An alphavirus structural protein expression cassette, comprising a promoter operably linked to a nucleic acid sequence encoding alphavirus structural proteins, wherein said nucleic acid sequence comprises a sequence encoding glycoprotein E2, and wherein said sequence encodes an amino acid substitution at E2 residue 160, as compared to wild-type.

13. An alphavirus packaging cell, comprising a host cell and an alphavirus structural protein expression cassette according to claims 11 or 12.

14. An alphavirus producer cell, comprising a packaging cell according to claim 13 and a vector selected from the group consisting of an alphavirus RNA vector replicon, alphavirus vector construct, and a eukaryotic layered vector initiation system.

15. A recombinant alphavirus particle, comprising a particle produced from a producer cell line according to claim 14.

16. A recombinant alphavirus particle, comprising a particle produced from a packaging cell line according to claim 13.

~~Sub. D3~~
~~Sub. F2~~
17. A recombinant alphavirus particle which infects human dendritic cells, with the proviso that said recombinant alphavirus particle is not derived from ATCC # VR-2526.

18. A recombinant alphavirus particle which infects non-human dendritic cells, with the proviso that said recombinant alphavirus particle is not derived from a Venezuelan equine encephalitis virus or ATCC # VR-2526.

~~Sub. D4~~
19. The recombinant alphavirus particle according to claims 17 or 18 wherein said alphavirus is a Sindbis virus.

20. The recombinant alphavirus particle according to claim 19 wherein said alphavirus has an amino acid substitution at E2 residue 160, as compared to wild-type Sindbis virus.

~~Sub. D4~~
21. The recombinant alphavirus particle according to claims 17 or 18 wherein said alphavirus is a Semliki Forest virus.

22. The recombinant alphavirus particle according to claims 17 or 18 wherein said alphavirus is Ross River virus.

23. The recombinant alphavirus particle according to claim 17 wherein said alphavirus is a Venezuelan equine encephalitis virus.

24. A method for introducing a heterologous nucleotide sequence into cells, comprising infecting said cells with a recombinant alphavirus particle according to claim any one of claims 15 to 23, such that said heterologous sequence is introduced into said cell.

25. The method according to claim 24 wherein said heterologous sequence is a sequence that encodes a protein.

26. The method according to claim 25 wherein said protein is an antigen from a pathogenic agent.

27. The method according to claim 26 wherein said antigen is from a virus, bacteria, parasite, or fungus.

28. The method according to claim 26 wherein said antigen is from a cancerous cell.

29. The method according to claim 25 wherein said protein is selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, alpha-IFN, beta-IFN, gamma-IFN, G-CSF, and GM-CSF

30. The method according to claim 24 wherein said heterologous sequence is a ribozyme or antisense.

31. The method according to claim 24 wherein said cell is infected ex vivo.

32. The method according to claim 24 wherein said cell is infected in vivo.

33. The method according to claim 24 wherein said cell is a population of cells comprising dendritic cells.

34. The method according to claim 33 wherein said dendritic cells are human dendritic cells.

35. An alphavirus vector construct, comprising (a) a 5' promoter which initiates synthesis of viral RNA *in vitro* from cDNA, (b) a 5' sequence which initiates transcription of alphavirus RNA, (c) a nucleic acid molecule which operably encodes all four

alphaviral nonstructural proteins, (d) an alphavirus RNA polymerase recognition sequence; and (e) a 3' polyadenylate tract, wherein said nucleic acid sequence which operably encodes all four alphaviral nonstructural proteins contains a mutation in at least one nonstructural protein selected from the group consisting of a mutation in nsP1 residues 346, 441, 473, nsP2 residues 438, 622, 634, 715, nsP3 residues, 417, 456, 505, and nsP4 residue 266, as compared to wild-type.

36. A eukaryotic layered vector initiation system, comprising a 5' promoter capable of initiating *in vivo* the 5' synthesis of alphavirus RNA from cDNA, a sequence which initiates transcription of alphavirus RNA following the 5' promoter, a nucleic acid molecule which operably encodes all four alphaviral nonstructural proteins, an alphavirus RNA polymerase recognition sequence, and a 3' polyadenylate tract, wherein said nucleic acid sequence which operably encodes all four alphaviral nonstructural proteins contains a mutation in at least one nonstructural protein selected from the group consisting of a mutation in nsP1 residues 346, 441, 473, nsP2 residues 438, 622, 634, 715, nsP3 residues, 417, 456, 505, and nsP4 residue 266, as compared to wild-type.

37. An alphavirus RNA vector replicon capable of translation in a eukaryotic system, comprising a 5' sequence which initiates transcription of alphavirus RNA, a nucleic acid molecule which operably encodes all four alphaviral nonstructural proteins, an alphavirus RNA polymerase recognition sequence and a 3' polyadenylate tract, wherein said nucleic acid sequence which operably encodes all four alphaviral nonstructural proteins contains a mutation in at least one nonstructural protein selected from the group consisting of a mutation in nsP1 residues 346, 441, 473, nsP2 residues 438, 622, 634, 715, nsP3 residues, 417, 456, 505, and nsP4 residue 266, as compared to wild-type.